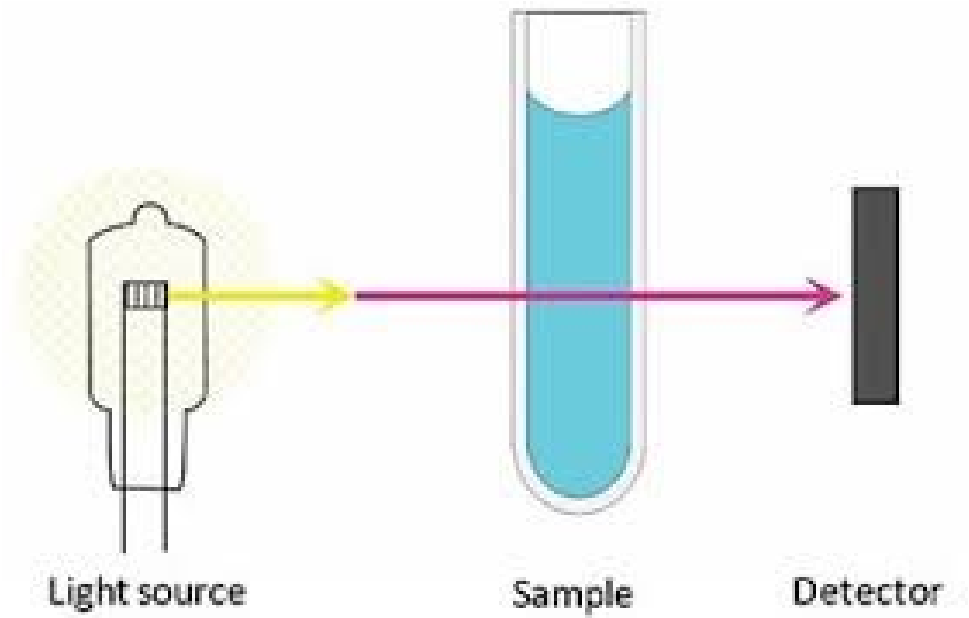
GENE2230 Laboratory Techniques

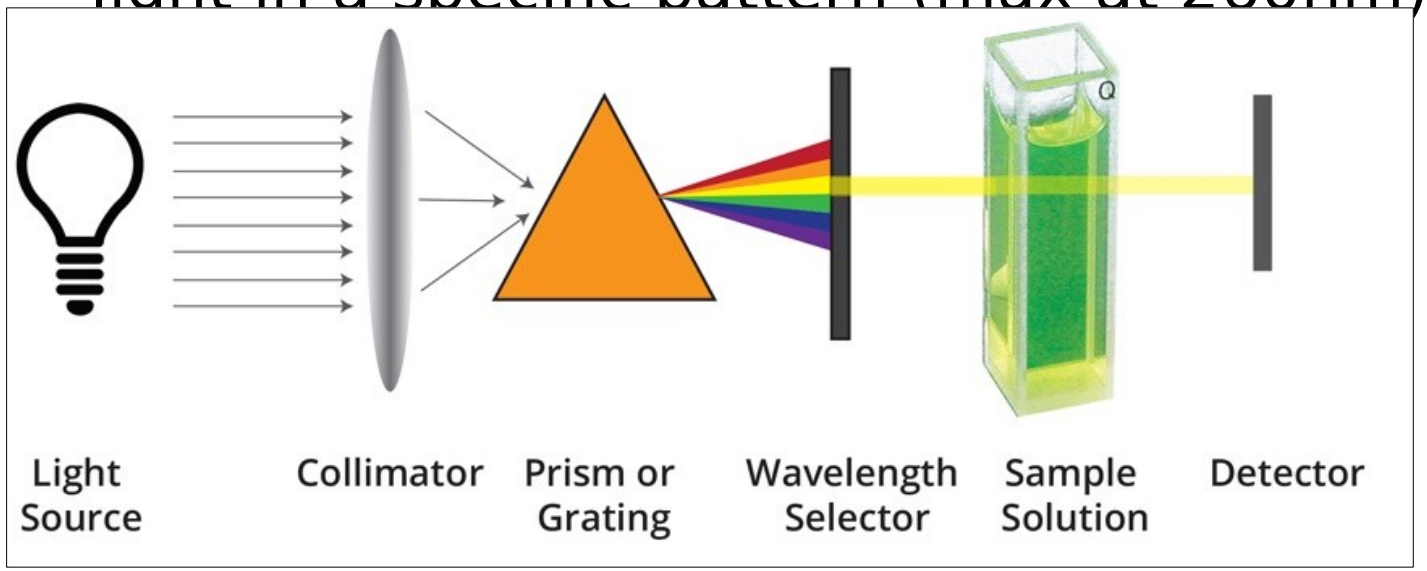
Nina McCarthy



Quantifying Nucleic Acids Using a Spectrophotometer

Spectrophotometry can determine the concentration and purity of nucleic acids in a sample

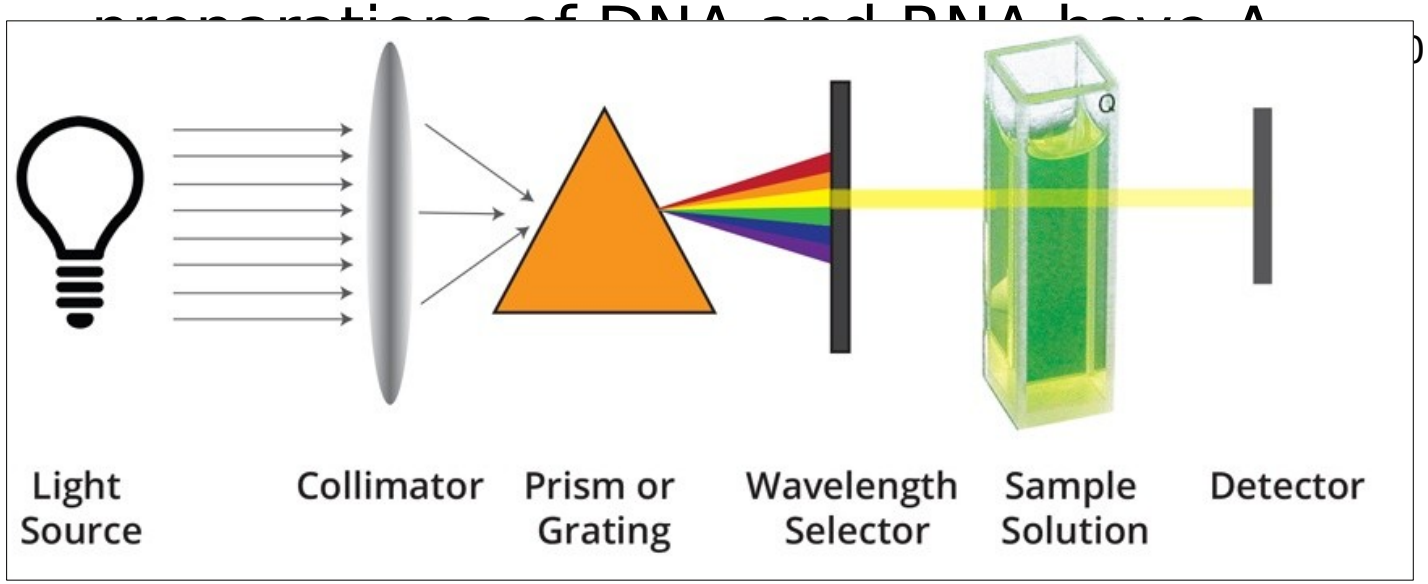
- Isolation of nucleic acids from biological tissues – ‘pure’ sample (uncontaminated by the other macromolecule classes present in cells: proteins, lipids and carbohydrates)
- Spectrophotometry can determine the **concentration and purity** of nucleic acids – based on the fact that nucleic acids absorb ultraviolet light in a specific pattern (max at 260nm)



- Sample is exposed to UV light at a specific wavelength.
- The amount of light that passes through the sample and hits the photodetector is measured.
- Less light = higher absorbance = higher optical

Spectrophotometry can determine the concentration and purity of nucleic acids in a sample

- **Concentration:** Amount of light absorbed (OD) at 260nm is proportional to DNA/RNA concentration. Conversion factor: for dsDNA, OD of 1 is equivalent to 50µg/ml.
- **Purity**: described using ratios of absorbance at different wavelengths. Ratio of the absorbance at 260 and 280 nm ($A_{260/280}$) is used to assess the purity of nucleic acids (proteins absorb at 280nm). Pure preparations of DNA and RNA have A ratios of 1.8 and 2.0,



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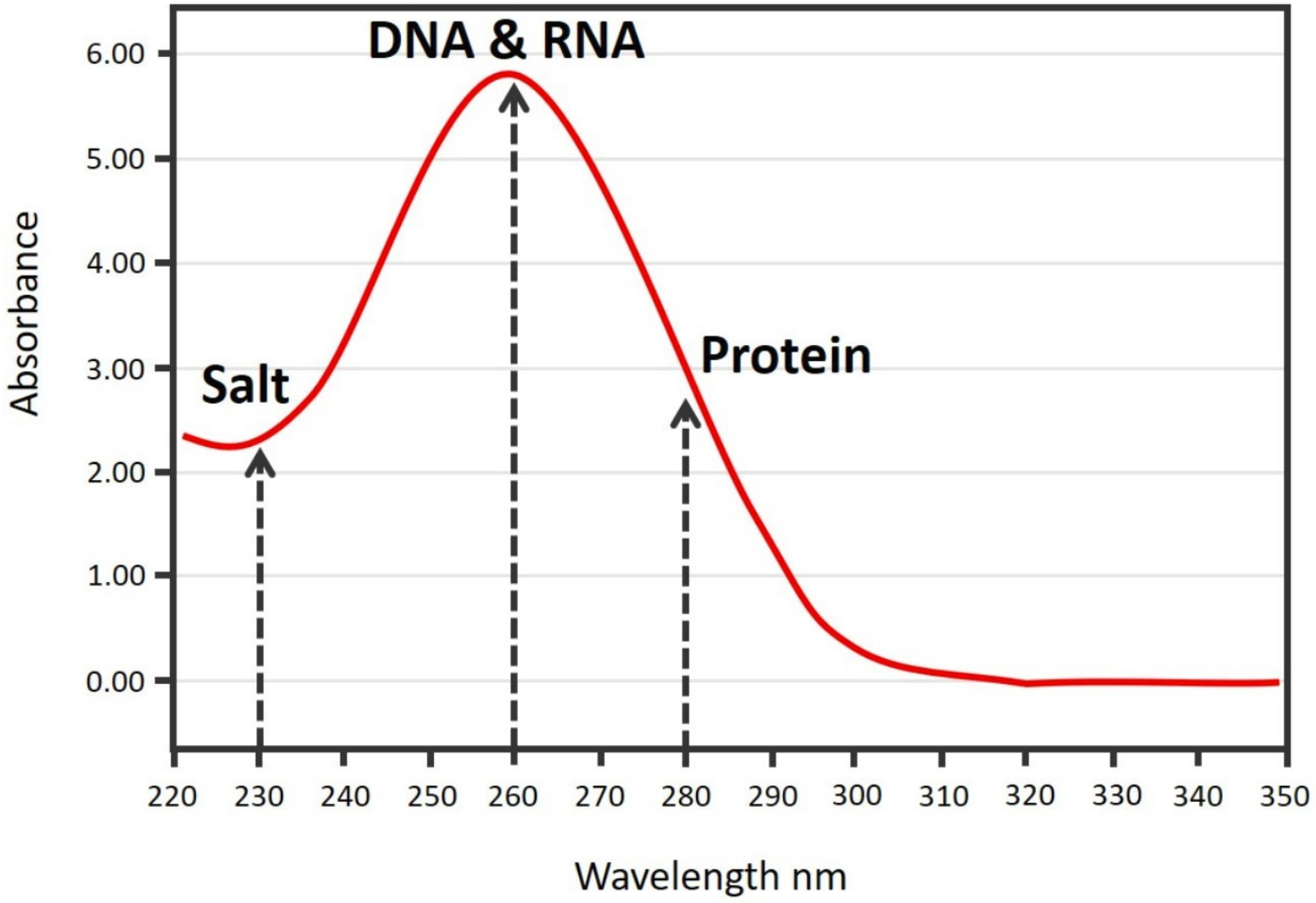
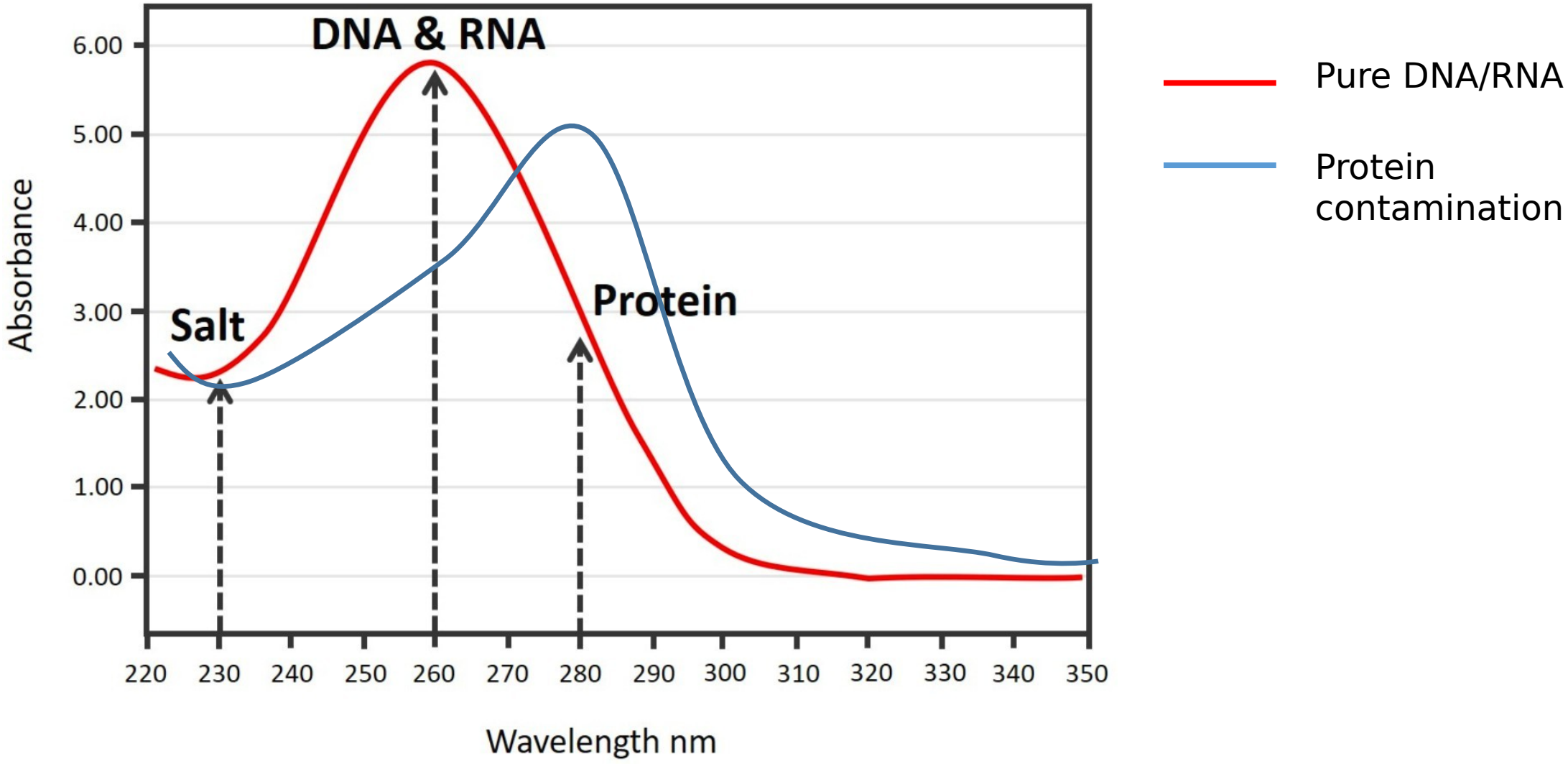
Absorbance curve for a DNA sample

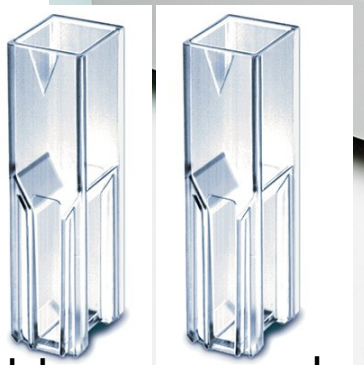


Absorbance curve for a DNA sample



Using a spectrophotometer

- If you have no idea how concentrated the DNA is, do a serial dilution (spectrophotometer only accurate at a certain absorbance range - eg OD 0.1 to 1.0).
- Prepare a ‘blank’ solution (everything but the DNA – water/buffer)
- Turn on the spec, clean the cuvettes and load with the blank
- Set the wavelength, place the blank in the cuvette holder, close the lid. Set the blank to 0.
- Remove the blank and put the experimental sample in the machine. Measure the absorbance.
- Repeat with successive wavelengths of light (re-blank).
- Empty and clean the cuvettes.



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Further resources:

- https://www.youtube.com/watch?v=pxC6F7bK8CU – how a spec works
- https://www.youtube.com/watch?v=qw2ZaUXgWHU – example of quantifying DNA

- https://www.wikihow.com/Do-Spectrophotometric-Analysis
- https://www.khanacademy.org/science/ap-chemistry/kinetics-ap/spectrophotometry-tutorial-ap/v/spectrophotometry-introduction